

# A review of laboratory results for bioaccessibility values of arsenic, lead and nickel in contaminated UK soils

SOHEL SAIKAT,<sup>1</sup> BOB BARNES<sup>2</sup> and DAVID WESTWOOD<sup>3</sup>

<sup>1</sup>*Environment and Human Health Science, Environment Agency, Howberry Park, Wallingford, Oxon, OX10 8BD, UK*

<sup>2</sup>*Integrated Catchment Science Group, Environment Agency, Olton, Solihull, B92 7HX, UK*

<sup>3</sup>*National Laboratory Services, Environment Agency, Rothley, Leicester, LE7 7NW, UK*

This paper presents the key findings of an Environment Agency's project aimed to ascertain what types of in vitro bioaccessibility methods are currently being used in the United Kingdom, what information is being reported and how they compare. Three samples of soil with elevated levels of arsenic, lead and nickel were collected from contaminated sites. The prepared and homogenised soils were sent as blind triplicate samples to 11 participating laboratories, of which 2 were from outside the UK. Analysis for total arsenic, lead and nickel content and the in vitro bioaccessible fraction was requested. An aqua regia extract was also prepared and sent with the soil samples for analysis. Whilst laboratory identification remains anonymous, codes A, B, C, D, E, F, G, H, J, K and L were assigned to individual laboratories. Three different types of in vitro methods were identified in the project producing bioaccessibility data. Each laboratory used the same bioaccessibility method for all three contaminants, irrespective of concentration or matrix. The results varied between laboratories and the variability is largely attributed to the difference in the in vitro methods used.

**Keywords:** In vitro, bioaccessibility, arsenic, lead, nickel, contaminated soil, laboratories, Z-score.

## Introduction

In large areas of the United Kingdom, natural arsenic levels in soil exceed the UK's soil guideline value (SGV) described for certain land uses.<sup>[1]</sup> SGVs represent "intervention values", which indicate that soil concentrations above this value at a particular site might pose an unacceptable risk to the health of the public and that further investigation and/or remediation is required.<sup>[1,2]</sup> The sources of such elevated levels are not necessarily anthropogenic in origin, but mainly relate to the soil's origins. Like most assessment criteria, SGVs that are derived using the Contaminated Land Exposure Assessment (CLEA) Model include assumptions that the contaminant of interest might be absorbed into the human body from soil to the same extent as from other media. Examples of other media could be water, diet, etc. used in studies to derive oral Health Criteria Values (HCVs).<sup>[3]</sup> These assumptions, however, might not be true; for instance, contaminants may be tightly bound to soil because of their sequestration in

soil, and this binding may lead to smaller quantities of contaminants being available for absorption by humans. For metals, for example, this may be due to their presence in discrete mineral phases (for example arsenic with iron and sulphides) and/or their chemical bonding to soil minerals. Hence, obtaining information on site-specific bioavailability of the contaminant in the soil relative to that of the medium used in the study (e.g., toxicological study), might reduce uncertainty and strengthen the risk assessment process.<sup>[4]</sup>

In human health risk assessment, two operational definitions of bioavailability have been used—absolute and relative. Bioavailability or absolute bioavailability refers to the fraction of the contaminant that can be absorbed by the body through the gastrointestinal system, pulmonary system or skin. In risk assessment of land contamination, it can simply be the ratio of the amount of contaminant absorbed by the body compared to the amount ingested.<sup>[5]</sup> Relative bioavailability is the comparison of absolute bioavailabilities of different forms of a contaminant or for different exposure media containing the contaminant. Relative bioavailability is important in the risk assessment of land contamination, where matrix effects can substantially alter the bioavailability of soil-bound contaminant to the form of the contaminant and dosing medium used in the critical toxicity study.<sup>[6]</sup>

Address correspondence to Sohel Saikat, Environment and Human Health Science, Environment Agency, Evenlode House, Howberry Park, Wallingford, Oxon, OX10 8BD, UK; E-mail: sohel.saikat@environment-agency.gov.uk

Determination of bioavailability requires direct tests with humans, or suitable animal models as surrogates for humans. The use of humans for bioavailability measurement is unlikely to be feasible. The routine use of animals is also challenging in terms of cost, time, facilities and ethical issues. Therefore, research efforts, over the last 10 years, have been directed towards developing suitable in vitro methods for measuring bioaccessibility as a surrogate of relative bioavailability. Bioaccessibility (or oral bioaccessibility), in human health risk assessment, describes the fraction of a contaminant released/dissolved from soil in an in vitro study. Most in vitro test methods are aimed at measuring the release/solubilisation of a contaminant from soil into an extraction solvent that aims to resemble human gut fluid. The principle, underlying in vitro method development, is that uptake (i.e., bioavailability) of a contaminant depends on the rate and extent of its dissolution (i.e., bioaccessibility) in the gut.<sup>[5,7]</sup> Current research efforts in the UK and other countries of Europe and North America are mainly centred around addressing the issues such as validation, reproducibility, robustness of in vitro methods for regulatory acceptance in risk assessment.<sup>[2–4]</sup>

As part of the Environment Agency's ongoing research programme on oral bioaccessibility testing, a project was commissioned to ascertain what types of in vitro bioaccessibility methods are currently being practised in the UK and what information is being reported and how it compares. As none of the in vitro methods has been approved by regulators in the UK, the project did not specify any particular method to apply in the study. The aim of this article is to present the approach undertaken in the project, and a summary of some key findings based on initial observations of the experimental part of the project. Although the aim of the project was not an inter-laboratory exercise, it has been organised as such to ensure consistency and that the same samples have been circulated to all participating laboratories.

## Materials and methods

### Sample collection, preparation and distribution

Soil was collected at three locations in England where levels of the metals in question were believed to be greater than their respective SGVs for residential housing with gardens (i.e., SGVs for arsenic, lead and nickel of 20, 450 and 50 mg/kg dry weight, respectively<sup>[1,8,9]</sup>). A portable X-ray fluorescence (XRF) detector was used at each sample location to determine whether metal concentrations were above the respective SGVs. The soil at each location was described in accordance with recognised procedures.<sup>[10]</sup>

The three soils sampled were selected (from areas of London, Bristol and Newcastle), mainly on the basis of the metal concentrations determined by XRF. However, physical observations of each soil (for example, with respect to

grain size, organic matter content, etc.) were also considered. A brief description of the selected soils is provided in Table 1.

From each site, composite sampling (within 1 m distance of each sampling point) was conducted to collect approximately 20 kg of top soil, after carefully removing vegetation and other extraneous material. Within 24 hours of collection, samples were placed in large, clean plastic bags, placed into a protective container and transferred to the UK Laboratory of Government Chemist (LGC) for preparation and distribution to the laboratories.

Each sample was air-dried at 25°C, gently dis-aggregated, sieved to <250 µm, thoroughly mixed, and split into sub-samples, each of 25 g quantities. All sub-samples were gamma irradiated to inhibit any residual microbiological activity within each sub-sample and ensure a sterile sample was distributed. Homogeneity testing was carried out for each metal, on replicate analyses of  $3\sqrt{n}$  sub-samples, where  $n$  was the total number of sub-samples prepared for each sample, until a relative standard deviation of less than 5% was obtained. The sealed bottles were subsequently refrigerated pending despatch to participating laboratories.

Prior to commissioning this project, it was unknown how many laboratories in the UK offered in vitro bioaccessibility testing. A questionnaire survey was carried out to identify those laboratories undertaking in vitro bioaccessibility determinations and showing an interest in participating in this project as per the scope and objectives of the project. A total of 11 laboratories participated in the study of which 2 were from outside the UK, i.e., University of Colorado Geological Sciences Laboratory in the United States and RIVM in the Netherlands.

The three UK contaminated soils were sent to participating laboratories as triplicate samples; however, laboratories were not informed of this. An aqua regia extract of a prepared soil was also distributed for analysis with a request to determine soluble arsenic, lead and nickel concentrations.

**Table 1.** Selected physico chemical characteristics of soils 1, 2 and 3 based on field observations

Sample	Approximate chemical concentration by portable XRF (mg/kg)		Physical characteristics of samples
Soil 1	Arsenic	45	Fine sand, silt with presence of ash
	Lead	90–135	
	Nickel	150–220	
Soil 2	Arsenic	140–230	Clay-silt with some organic matter
	Lead	2500–8500	
	Nickel	80 ± 30	
Soil 3	Arsenic	16000*	Medium dense amorphous black organic silt with occasional fine to medium gravel
	Lead	77000*	
	Nickel	Not determined	

\*— above instrument calibration.

All samples were randomly numbered from 1 to 100 and despatched to laboratories, along with the prepared aqua regia extract.

Laboratories were informed of the sample preparation stages and requested to undertake analysis for total metal concentration and in vitro bioaccessibility of arsenic, lead and nickel. Laboratories were to use their normal routine methods offered as part of their current practices in in vitro bioaccessibility determination and to report their results in their usual format.

Although no information was available as to the nature of in vitro bioaccessibility methods used within UK laboratories prior to this project, the samples were prepared and distributed according to the principles, practices and procedures adopted by the CONTEST soil proficiency-testing scheme.<sup>[11]</sup> These procedures are based on internationally recognised procedure for conducting inter-laboratory comparisons.<sup>[12,13]</sup>

### Data analysis

To assess the comparability of the results generated and to ascertain whether they are consistent with each other, a z-score approach was adopted using the scheme used by the CONTEST soil proficiency-testing scheme. The value of the z-score was calculated using the equation:

$$z - score = \frac{\text{laboratory value} - \text{assigned value}}{\text{established standard deviation}}$$

where, the assigned value is the median value of all results from all laboratories for a particular sample; and the established standard deviation is a percentage of the median value. The established standard deviation was chosen on the basis of the complexity of the analysis and the concentration level of the metal in the matrix. The values adopted in the CONTEST soil proficiency-testing scheme were used in this exercise for the aqua regia extract solution and the total metal determinations and these values ranged between 7.5%, 10%, 12.5% and 15% depending on the matrix, expected concentration and element. For the bioaccessibility determinations, a value of 20% of the median value has been used as the established standard deviation for all metals due to the complexity of in vitro testing.

Using this approach, it is recognised that the median value of laboratory results need not necessarily represent the true or most accurate value of a particular soil, i.e., the assigned value. However, the median value, instead of a mean value, is less prone to effects from outlying results that are not part of the normal distribution of results. None of the UK soil samples has undergone in-vivo testing and hence the true or accepted bioavailability value is unknown, thus the median value of laboratory results may not necessarily represent the true or actual bioaccessibility fraction. In view of this, results cannot be regarded as satisfactory

or unsatisfactory, but only whether they are consistent with each other.

In normal circumstances where the true or accepted value is known, Z-score values can be used according to the following scale:

$ Z  \leq 2$	satisfactory
$2 <  Z  < 3$	questionable
$3 \geq  Z $	unsatisfactory

Highlighted in black, Figures 1–3 show results that are inconsistent with those results reported by other laboratories, i.e., where z-score values are greater than 3 or less than –3.

### Results and discussion

In the expression of results, participating laboratories were kept anonymous. Codes A, B, C, D, E, F, G, H, J, K and L were assigned to individual laboratories.

#### Types of in vitro methods used by laboratories

In the present study, most UK laboratories used a slightly modified version of a method described by Ruby et al.<sup>[14]</sup> (Method 1 in Table 2). Modifications included adjusting the pH in the intestinal phases and not performing the test in anoxic conditions with argon gas. The method developed by Ruby et al. is known as a “physiologically based extraction test” (PBET). It comprises two extraction phases, i.e., stomach and intestine, where enzymes and organic acids are added to mimic gastric and small-intestinal fluids. The chemicals used in the gastric fluid include pepsin, sodium malate, sodium citrate, lactic acid and deionised water. In addition, bile salts, pancreatin and sodium bicarbonate are added to mimic intestinal fluid.

A slightly different physiologically based method to the PBET method (for pH in stomach and intestine steps, solid/liquid ratio) was also used by a laboratory (Method 3 in Table 2). In contrast to physiologically based methods, two laboratories produced bioaccessibility data with a method based on a simple buffered acid (0.4 M glycine, pH 1.5; Method 3 in Table 2). This method does not use any enzymes or organic acids to mimic the gut fluid and also does not include an intestine phase. Another laboratory, which does not undertake routine bioaccessibility determinations, produced results based on the extraction procedure using dilute EDTA and acetic acid solutions (Method 4 in Table 2). The results of this laboratory, however, were not included in the statistical treatment and are shown in the figures for comparison purposes only.

Although individual laboratories differed from each other for using different in vitro methods, each laboratory used the same method for all bioaccessibility determinations of arsenic, lead and nickel.

**Table 2.** Key characteristics of in vitro methods used for bioaccessibility determination

Method	Lab	Types	Temp (°C)	SP	IP	pH		Liquid/Solid ratio
						SP	IP	
1	A, C, D, E, F, G, J	Physiologically based	37	✓	✓	2.5	7	100/1
2	H, K	Simple buffered acid	37	✓	×	1.5	×	100/1
3	L	Physiologically based	37	✓	✓	1.1	5.5	<sup>1</sup> Stomach 37.5/1 intestine 97.5/1
4	B	EDTA or CH <sub>3</sub> COOH solution	RT	None		i) pH 7 (EDTA solution) ii) 0.43 M CH <sub>3</sub> COOH solution		i) 10/1 (EDTA extraction) ii) 40/1 (CH <sub>3</sub> COOH extraction)

S P is stomach phase.

IP is intestinal phase.

RT is room temperature.

1: When the sample size is 0.06 g, the liquid/solid ratio for the stomach phase is 375/1 and for the intestine phase is 975/1.

### Expression of bioaccessibility results

Regardless of the types of in vitro methods used, laboratories expressed their results as percentage (%) oral bioaccessibility using the equation:

$$\% \text{bioaccessibility} = \frac{\text{metal concentration in extract solution} \times 100}{\text{total metal concentration in soil}}$$

If recourse to the total metal concentration was not to be taken into account, then bioaccessibility was calculated as the soluble metal concentration in the extract solution.

Thus,

$$\text{Bioaccessibility} = \text{soluble metal concentration in extract solution mg/L}$$

This concentration was then expressed on a unit weight basis, i.e., mg/kg.

Expressing the bioaccessibility results in percentage terms requires a total metal concentration to be determined. For this determination, all laboratories, except one, used an aqua regia extract technique for extracting the metals from the soils. It has been reported that depending on the particular matrix, methods involving aqua regia extraction can produce results that may be as low as 30% of the true or accepted total value.<sup>[15]</sup> For this project, only one laboratory used an XRF technique for determining total metal concentrations, a technique that may intuitively produce higher results than for an aqua regia extraction technique. Irrespective of the matrix, metal or metal concentration determined, all laboratories used a single technique for determining the three metal concentrations, despite the fact that this may not be appropriate for all metals determined and may not be optimised for each metal in each matrix.

All participating laboratories, except one, that included both stomach and intestine phases in their methods, considered the highest fraction, regardless of phases, as their

bioaccessibility estimates for all three metals (arsenic, lead and nickel). Details of different phases that were reported by various laboratory are provided elsewhere.<sup>[16]</sup> This precautionary approach is to ensure a conservative estimate of bioaccessibility, which in turn is to make a conservative prediction of bioavailability for use in risk assessments. These highest fractions were, however, not necessarily restricted to a particular phase and were derived from either stomach or intestine phases. One laboratory, in contrast, recommended the use of only the results of the intestine phase as their estimate of bioaccessibility. This laboratory indicated that the absorption of chemicals, e.g., lead, takes place in the intestinal compartment but not in the stomach, therefore the use of intestine data as the bioaccessibility estimates have a more physiological basis, and are more scientifically justified.<sup>[17]</sup>

### Extract solution

All laboratories reported values for arsenic, lead and nickel, respectively for the aqua regia extract solution provided. These results are summarised in Table 3. Generally, the results are in agreement and no outliers are indicated by Dixon's test. It is, however, noted that laboratory D produced the lowest result (1.7 mg/L) for arsenic, and the highest result (320 mg/L) for lead in the aqua regia extract solution. These values are not consistent with the other results when compared using a z-score approach, i.e., z-score values of -3.93 and 3.67, respectively. The results generally indicate that laboratories experienced few difficulties analysing all three metals in the aqua regia extract solution, irrespective of the methodologies used.

### Total metal concentrations

Most laboratories reported concentrations for arsenic, lead and nickel in the four soils distributed. From the summary

**Table 3.** Summary of results for aqua regia extract solution and total concentrations of arsenic, lead and nickel in soils 1, 2 and 3

	<i>Arsenic</i>		<i>Lead</i>		<i>Nickel</i>	
	<i>Median</i>	<i>RoSD</i>	<i>Median</i>	<i>RoSD</i>	<i>Median</i>	<i>RoSD</i>
Extract solution (mg/l)	<b>3.34</b>	0.6	<b>251</b>	9	<b>0.9</b>	0.1
Number of laboratories	11		11		10	
Range (mg/l)	1.7–3.88		220–320		0.74–1.08	
Total metal concentration in test soils (mg/kg)						
Soil 1	<b>112</b>	24	<b>85</b>	21	<b>62</b>	13
Number of laboratories	10		10		9	
Mean range (mg/kg)	81–129		64–155		36–135	
Soil 2	<b>120</b>	22	<b>8124</b>	605	<b>31</b>	7
Number of laboratories	10		10		9	
Mean range (mg/kg)	96–143		7083–9647		21–38	
Soil 3	<b>10307</b>	1723	<b>75478</b>	30924	<b>43</b>	9
Number of laboratories	10		10		9	
Mean range (mg/kg)	8793–12988		22150–10000		27–61	

The median value is obtained from the means of all laboratory results.  
RoSD is the robust standard deviation.

of results presented in Table 3 for total metal concentrations, it appears that for the different soil matrices, the analysis of arsenic posed few problems leading to no z-score values greater than 3 or less than –3. Good agreement was observed for all laboratories, as shown by the comparison of the mean and standard deviation values, and the median and robust standard deviation values, at the levels determined despite different methodologies being used in all laboratories. This is in contrast with the results for lead and nickel. For lead, apart from soil 2 where the analysis seems satisfactory for all laboratories despite different methodologies, difficulties were experienced with soils 1 and 3. Difficulties with nickel for all soils are also apparent as the results show wide variation, which is in contrast with the nickel results for the aqua regia extract solution. Whether this situation is a reflection of poor laboratory performance or of the different methodologies, matrix or concentration levels determined is not clear.

#### *In vitro* bioaccessibility

As defined, the % bioaccessibility requires knowledge of the total metal concentration of the soil; however, bioaccessibility expressed as mg/kg does not. As bioaccessibility expressed as mg/kg is not dependent on knowledge of the total metal concentration, direct comparison of results could be undertaken. For this reason, and as the project is yet to evaluate all the results fully in consultation with participating laboratories, only the bioaccessibility results expressed as mg/kg have been discussed.

In the following sections, results obtained for arsenic in soil 1, lead and nickel in soil 2 and lead in soil 3 are presented. In Figures 1 to 4, bioaccessibility results of participating laboratories are compared, where results, as shown by z-score values being greater than 3 or less than –3, are shown in black. Not included in the statistical treatment

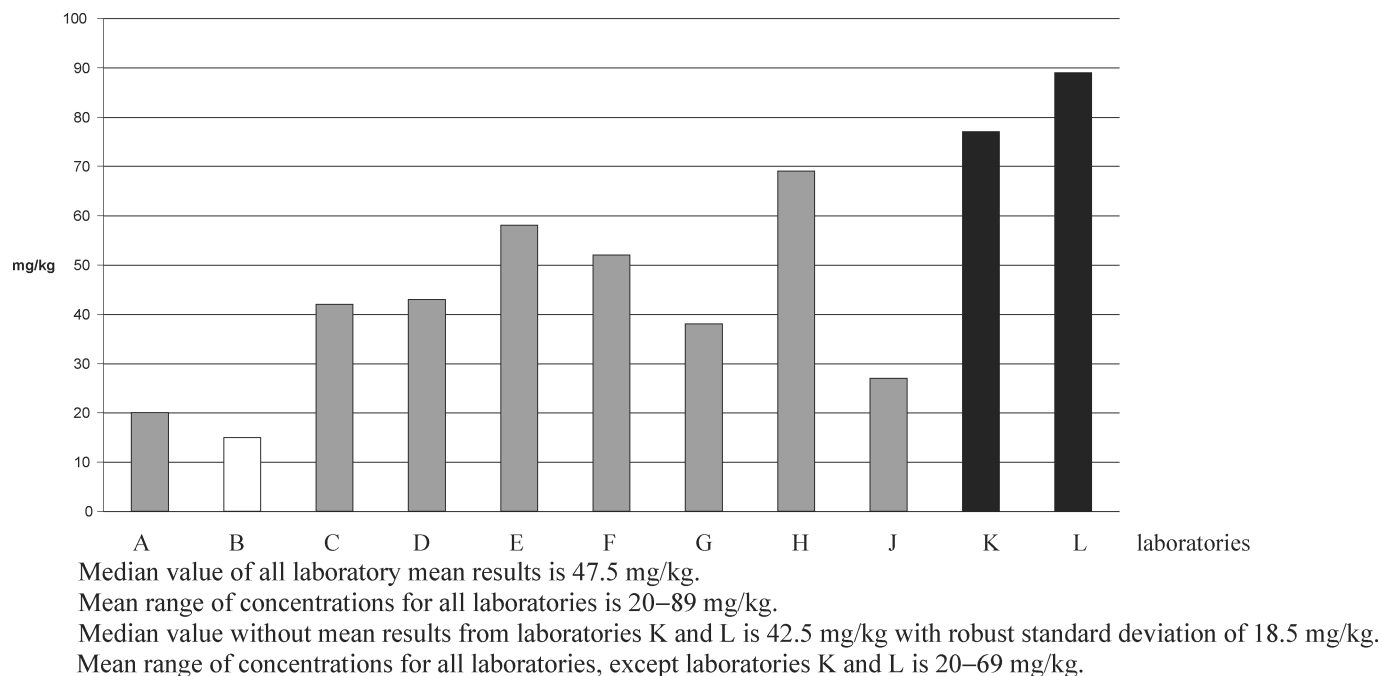
are the results from laboratory B, which as explained earlier did not use a bioaccessibility test method, *sensu stricto*, are shown for comparison purposes only (in white). It is, however, known that extraction results based on dilute EDTA and acetic acid solutions are, sometimes, sent to local authorities in the UK as part of the risk assessment process of contaminated land. <sup>[18]</sup>

#### *Soil 1*

For arsenic, reasonably good agreement between the laboratories is observed (mean bioaccessibility values between 20 and 89 mg/kg) with a median value of all results of 47.5 mg/kg. Two laboratories (i.e., laboratories K and L) reported mean values (77 and 89 mg/kg) deemed inconsistent with the other results, based on the z-score values of 3.1 and 4.4 respectively (Fig. 1). The median value without mean results from laboratories K and L is 42.5 mg/kg with robust standard deviation of 18.5 mg/kg. The mean range of concentrations for all laboratories, except laboratories K and L is 20–69 mg/kg. For comparison purposes only, laboratory B reported a mean EDTA extraction value of 15 mg/kg.

#### *Soil 2*

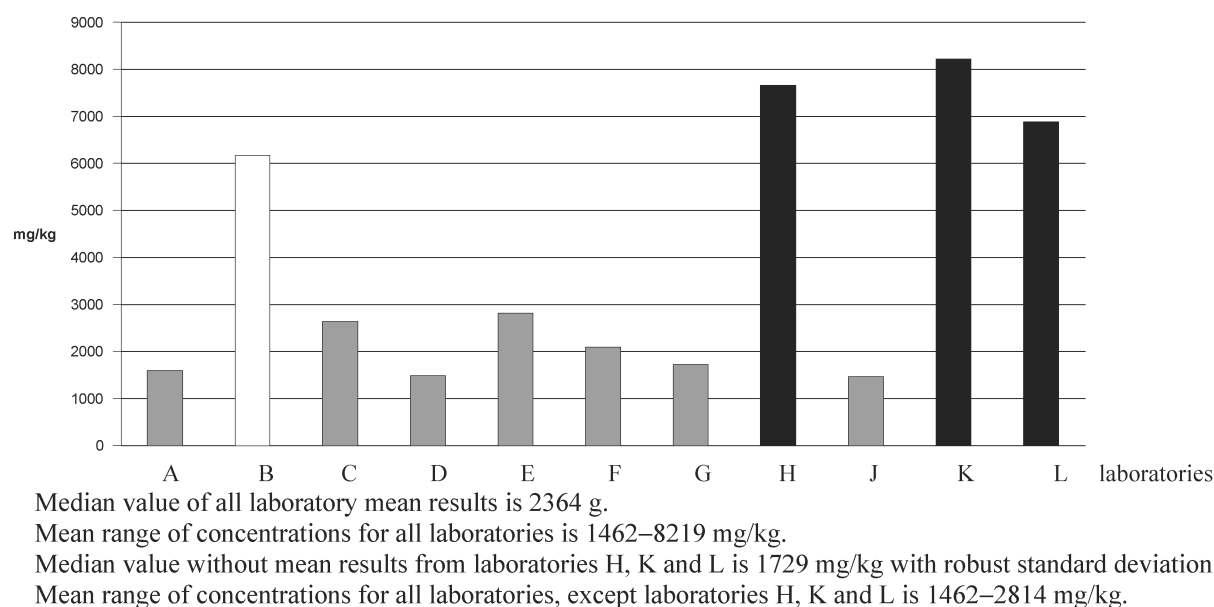
For lead in soil 2, the results appear to fall into 2 main groups (Fig. 2). For one group (laboratories A, C, D, E, F, G and J) the mean range varies between 1462–2814 mg/kg and for the other group (laboratories H, K and L) the mean range varies between 6887–8219 mg/kg. The results from this second group are deemed inconsistent with the other results, based on the z-score values of 11.2, 12.4 and 9.6, respectively. However, since a consensus value has been used in the calculation of the z-score, the true result is not known. The median value of all laboratory mean results is



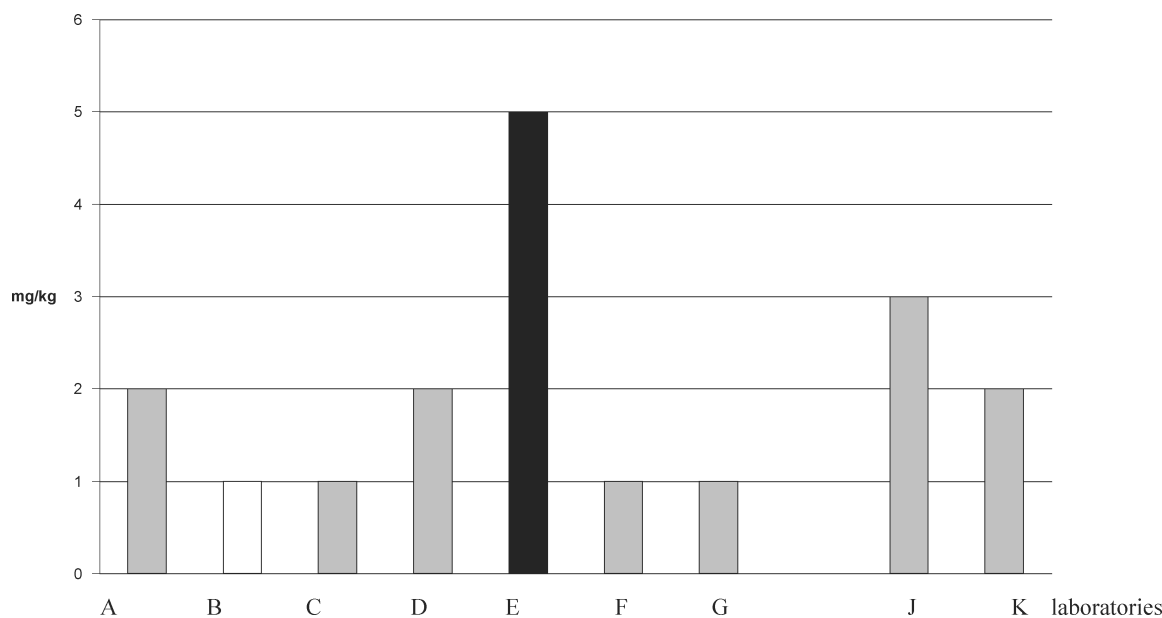
**Fig. 1.** Arsenic bioaccessibility values (mg/kg) in soil 1.

2364 mg/kg. The mean range of concentrations for all laboratories is 1462–8219 mg/kg. The median value without mean results from laboratories H, K and L is 1729 mg/kg with robust standard deviation of 396 mg/kg. For comparison purposes only, laboratory B reported a mean EDTA extraction value of 6203 mg/kg.

For nickel, a lower range of bioaccessibility results are reported for soil 2 (Fig. 3). The results, in general, varied between 1–5 mg/kg. For comparison, a mean EDTA value of 1 mg/kg is shown by laboratory B. Using the z-score approach, the result from laboratory E is deemed inconsistent with results from the other laboratories.



**Fig. 2.** Lead bioaccessibility values (mg/kg) in soil 2.



\* Nickel was not analysed by laboratory H and L.

Median value of all laboratory mean results is 2 mg/kg.

Mean range of concentrations for all laboratories is 1–5 mg/kg.

Median value without mean result from laboratory E is 2 mg/kg with robust standard deviation of 1.5 mg/kg.

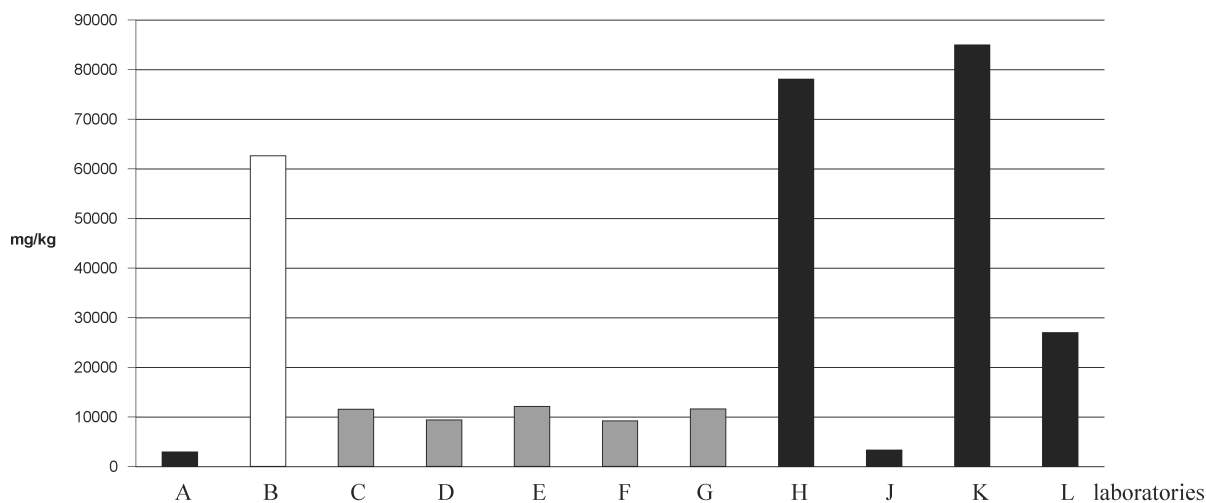
Mean range of concentrations for all laboratories, except laboratory E is 1–3 mg/kg.

**Fig. 3.** Nickel bioaccessibility values (mg/kg) in soil 2.

### Soil 3

For lead, three groups of data are apparent as shown in Figure 4: one group of 5 laboratories showing mean values (between 9204–12141 mg/kg) and another

group of 2 laboratories with mean values much lower (about 3000 mg/kg). Three laboratories show mean values between 27378–86890 mg/kg. For comparison, laboratory B reports a mean EDTA extraction value of 66100 mg/kg.



Median value of all laboratory mean results is 11591.5 mg/kg.

Mean range of concentrations for all laboratories is 2920–84979 mg/kg.

Median value without mean results from laboratories A, H, J, K and L is 11573 mg/kg with robust standard deviation of 842 mg/kg.

Mean range of concentrations for all laboratories, except laboratories A, H, J, K and L is 9204–12141 mg/kg.

**Fig. 4.** Lead bioaccessibility values (mg/kg) in soil 3.

Based on the overall spread of bioaccessibility results obtained for the soils 1, 2 and 3 for arsenic, lead and nickel, laboratories can be approximately categorised into 2 broad groups, although exceptions are noted. This categorisation, however, excludes laboratories A and J, as their results, in most cases, do not fall into any of the 2 groups, and also laboratory B, as the results from this laboratory are not regarded as bioaccessibility results and are shown for comparison purposes only. Group 1 comprises laboratories C, D, E, F and G, and group 2, laboratories H, K and L (Figs. 1–4).

Laboratories within group 1 tend to produce a lower range of results. For soil 1, for example, group 1 produces arsenic bioaccessibility results that range between 38 and 58 mg/kg, whereas group 2 results range between 69 and 89 mg/kg. Similar observations can be made with results for other soils and metals.

An alignment of the results into these two broad categories probably reflects the types of in vitro methods used by the laboratories. The laboratories within group 1, producing relatively low bioaccessibility results, use methods similar to that reported by Ruby et al.,<sup>[14]</sup> shown as Method 1 in Table 2. The methods used by laboratories in group 2, although, having generic similarities (e.g., temperature, mixing rate) to methods used by laboratories in group 1, exhibit differences in some key test conditions (e.g., pH, composition of extraction fluid). The laboratories within group 2 use Method 2 or 3 in Table 2. The higher bioaccessibility results obtained for laboratories in group 2 might indicate that the methods used by these laboratories are more efficient and/or aggressive in dissolving soil contaminants under the test conditions used.

It should be recalled that the approach taken does not allow for evaluation of the trueness of the methods. Furthermore, it should be noted that methods for bioaccessibility test are operationally defined and thus expected to produce different results with different methods applied. Details of the precision of individual methods within this study are available elsewhere.<sup>[16]</sup>

## Conclusions

The following main conclusions could be drawn from the study:

- (i) There are two main types of in vitro methods used by UK laboratories to produce bioaccessibility data for arsenic, lead and nickel: the modified method of Ruby et al.<sup>[13]</sup> commonly known as a PBET method, and a method based on a simple buffered acid solution (0.4M glycine, pH 1.5).<sup>[19]</sup> Individual laboratories use the same method for all determinations of bioaccessibility for all three contaminants.
- (ii) For this study, most of the UK laboratories undertaking in vitro bioaccessibility testing used aqua regia as

the extraction solution in the determination of total metal concentrations. Depending on the matrix, aqua regia may not be able to extract all of the metal present in a soil. Hence, the expression of bioaccessibility, as a percentage of the total concentration, based on an aqua regia extraction of the metal, should take this into account.

- (iii) Bioaccessibility results expressed as mg/kg should be free from the influence of total metal concentrations. However, the results of the present study show wide variability between laboratories for all three metals. Discounting the uncertainty in each determination, this variability is likely to be attributed to the differences in the in vitro methods used. As none of the UK soils have undergone into any in-vivo study, it is not meaningful to comment on any method as being truer than any other.
- (iv) For all three soils, the inter-laboratory comparison using the z-score approach indicates that arsenic presents fewer problems compared to lead and nickel determinations.
- (v) Analysing the same soil, using different in vitro methods, results in different bioaccessibility values being reported.

A full report on the project is being prepared and it is anticipated to be available early in 2007.<sup>[16]</sup>

## Disclaimer

The views expressed in this paper are not necessarily state or reflect those of the Environment Agency. Reference herein to any specific method, process or service does not necessarily constitute or imply its endorsement or recommendation by the Environment Agency.

## References

- [1] Defra and Environment Agency. *Soil guideline values for arsenic contamination*. R&D publication SGV 1, 2002; 14 pp.
- [2] Environment Agency. *Environment Agency's Science Update on the use of bioaccessibility testing in risk assessment of land contamination*. In ([www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=\\_e](http://www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=_e)).2005.)
- [3] Environment Agency. Report on the International Workshop on the Potential Use of Bioaccessibility Testing in Risk Assessment of Land Contamination; Saikat, S. Ed.; 2005, Science Report SC040054. In ([www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=\\_e](http://www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=_e)).
- [4] Saikat, S. *Bioavailability/bioaccessibility testing in risk assessment of land contamination: a short review*. UK Health Protection Agency Chem. Hazards Poisons Report. 2005, Issue 6. In ([www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=\\_e](http://www.environment-agency.gov.uk/subjects/landquality/113813/1283985/?version=1&lang=_e)).
- [5] US EPA. *Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using In vivo and In vitro methods*; Office of



- Solid Waste and Emergency Response, US EPA, Washington DC, 2005.
- [6] NFESC (Naval Facilities Engineering Service Centre) User's Guide UG-2041-ENV, 2000. *Guide for incorporating bioavailability adjustments into human health and ecological risk assessments at US Navy and Marine Corps facilities* (Part 2: Technical Background Document for Assessing Metals Bioavailability); Prepared by Battelle and Exponent, Washington, DC, 2000.
- [7] Rodriguez, R.R.; Basta, N. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and media. *Environ. Sci. Technol.* **1999**, *32*, 642–649.
- [8] Defra and Environment Agency. Soil Guideline Values for Lead Contamination. R & D publication SGV 10, 2002; 20 pp.
- [9] Defra and Environment Agency. Soil Guideline Values for Nickel Contamination. R & D publication SGV 7, 2002; 15 pp.
- [10] British Standards Institution. *Code of practice for site investigations*. BS5930:1999.
- [11] CONTEST Contaminated Land Proficiency Testing Scheme Protocol, 6th Edition, March 2005, LGC Teddington, Middlesex, TW11 0LY.
- [12] ISO/IEC Guide 43-1: *Proficiency testing by inter-laboratory comparisons—Part 1—Development and operation of proficiency testing schemes*. International Organisation for Standardisation/International Electrotechnical Commission, 1997.
- [13] ILAC-G13: *Guidelines for the requirements for the competence of providers of proficiency testing schemes*. International Laboratory Accreditation Cooperation, 2000.
- [14] Ruby, M.V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C.M. Estimation of lead and arsenic bioavailability using a physiologically based extraction test, *Environ. Sci. Technol.* **1996**, *30*, 422–430.
- [15] Environment Agency NCAS report (NCAS/TR/2002/023) *Trace metal analysis of environmental solid matrices*. 2002.
- [16] Environment Agency. Inter-laboratory comparison of in vitro bioaccessibility measurements for arsenic, lead and nickel in soil, 2007: In press.
- [17] Oomen, A.G.; Brandon, E.F.A.; Swartjes, F.A.; Sips, A.J.A.M. How can information on oral bioavailability improve human health risk assessment for lead-contaminated soils? RIVM report 711701042/2006, 2006.
- [18] Environment Agency. Questionnaire survey on the use of in vitro bioaccessibility in human health risk assessment. Science Report SC040060/SR1, 2006.
- [19] Drexler, J.W. An in vitro method that works! a simple, rapid and accurate method for determination of lead bioavailability. In *EPA Bioavailability Workshop*, Durham, NC., August, 1998.